Introduction of WAG, a Wheat AGAMOUS Homolog, Reduces Corolla Size in Torenia

journal or publication title	Bulletin of the National Institute of Floricultural Science
volume	3
page range	21-27
year	2003-10-31
URL	http://doi.org/10.24514/00001407

doi: 10.24514/00001407

原著論文

Introduction of *WAG*, a Wheat *AGAMOUS* Homolog, Reduces Corolla Size in Torenia

Ryutaro AIDA*,***, Koji MURAI**, Sanae KISHIMOTO* and Akemi OHMIYA*

(Received August 28,2003)

Summary

The function of a 1.1-kb transcript of *WAG*, a wheat *AGAMOUS* homolog, has not been clarified. To analyze its function, it was fused with the cauliflower mosaic virus 35S promoter and introduced into torenia (*Torenia fournieri*). Torenia transformants grew normally with regular plant height, size and shape of leaves, and development of inflorescence. However, the corolla was significantly smaller in 10 transformants out of 21. Generally, the vertical and horizontal diameters of the lip part of the corolla, as well as the length of the corolla (including the tube part), were all reduced in the smaller flowers, suggesting that the size of the corolla was reduced equally in all dimensions. Southern blot analysis showed the existence of the *WAG* gene in the genomes of these plants. We report that *WAG* reduces the size of the corolla in torenia.

Key Words: *AGAMOUS*, flower size, homeotic gene, MADS, torenia, transformation.

* Department of Genetics and Physiology
** Fukui Prefectural University, Matsuoka, Fukui 910-1195
*** Present affiliation: Department of Production

Introduction

The mechanisms of development of floral organs have been explained by the ABC model (Coen and Meyerowitz 1991). According to this model, the differentiation of 4 floral organs – sepals, petals, stamens, and carpels – is modulated by 3 classes of homeotic genes. Sepals are specified by class A genes in whorl 1 (the outermost whorl), petals by a combination of classes A and B in whorl 2, stamens by classes B and C in whorl 3, and carpels by class C alone in whorl 4 (the innermost whorl). In *Arabidopsis*, the class A genes are *APETALA1* (*AP1*) and *APETALA2* (*AP2*), the class B genes are *APETALA3* (*AP3*) and *PISTILLATA* (*PI*), and the class C gene is *AGAMOUS* (*AG*). Homologs of these genes have been isolated in many plant species and, in most cases, their expression is correlated with the development of floral organs (Theissen et al. 2000).

Homeotic change of the stamens into pistil-like structures (pistillody) occurs in cytoplasmic substitution lines of common wheat (*Triticum aestivum*) with *Aegilops crassa* cytoplasm (Tsunewaki 1989, Murai et al. 2002). The pistillody causes cytoplasmic male sterility. A wheat *AGAMOUS* homolog, *WAG*, is associated with the pistillody (Meguro et al. 2003). Meguro et al. reported that three copies of *WAG* exist in the wheat genome (chromosome 1A, 1B, and 1D) and that different combinations of the three copies produce a total of two different transcripts (1.1 kb and 1.3 kb). They also reported that only the 1.3-kb transcript, observed in pistils and pistil-like stamens, seemed to be involved in pistillody. The other transcript (1.1 kb), observed not only in the floral organs (lemma, palea, stamen, and pistil) but also in the leaves and stems, seemed not to be involved in pistillody. Although most of the *AG* homologs reported in different kinds of plant species are expressed only in the flower, the 1.1-kb-sized *WAG* transcript is also expressed in the vegetative organs. It would therefore be of great interest to know the function of the 1.1-kb-sized *WAG* transcript.

Ectopic expression of the *WAG* gene for the 1.1-kb transcript in a heterologous plant would give us hints to its function. Torenia (*Torenia fournieri*) seems a good plant material to use for investigating the function of floral homeotic genes, because it is easy to transform and can flower *in vitro* (Aida and Shibata 2001). We therefore fused the *WAG* coding region for the 1.1-kb transcript with the cauliflower mosaic virus 35S promoter and introduced it into torenia by *Agrobacterium*-mediated transformation. We then examined the characters of the transformed plants to determine the function of the *WAG* gene for the 1.1-kb transcript.

Materials and Methods

Vectors and *Agrobacterium*

Vector plasmids, pBIWAG-sense and pBIWAG-antisense, were constructed from pBI121 (Clontech, Palo Alto, CA, USA) by replacing the β-glucuronidase gene with the *WAG* gene for the 1.1-kb transcript (Meguro et al. 2003) in a sense or antisense orientation. *Agrobacterium tumefaciens* strain EHA105 was used for the experiments.

Plant materials and transformation

The experiments used a violet clonal laboratory line (Aida et al. 2000) selected from 'Crown Mix'. Transgenic plants were obtained by the *Agrobacterium*-mediated transformation system, as described previously (Aida and Shibata 2001). Transformants were grown in a closed greenhouse for the investigation.

Southern blot analysis

Total DNA was extracted from leaf tissue with a Phytopure plant DNA extraction kit (Amersham Pharmacia, Little Chalfont, Buckinghamshire, England) according to the manufacturer's instructions. About 10 μg of DNA digested with *Hin*d Ⅲ was electrophoresed in a 0.6% agarose gel and transferred to a positively charged nylon membrane (Roche Diagnostics,

Mannheim, Germany). Southern blot analysis was performed with the DIG-High Prime and DIG Luminescent Detection kits for nucleic acid (Roche Diagnostics). The coding region of the *WAG* gene for the 1.1-kb transcript was used as a probe. Blots were finally washed with 0.2 × SSC, 0.1% SDS, at 68 ℃ .

Flow cytometric analysis of nuclear DNA content

For the isolation of nuclei, pieces of leaf about 5 mm square were chopped up with a razor blade in a few drops of buffer A of a Partec High Resolution Staining Kit for Plant DNA Analysis (Partec GmbH, Münster, Germany). After 0.2 mL of buffer A had been added, the suspension with nuclei was filtered through a 30-μm mesh filter. About 1.5 mL of buffer B from the kit was added, and the intensity of fluorescence of the nuclei was immediately measured with a Partec PA flow cytometer.

Results and Discussion

Phenotypic changes in transformants

We obtained 21 transformants with the sense *WAG* and 12 with the antisense *WAG*. The transformants were potted in soil and allowed to flower in a closed greenhouse for transgenic plants. All the transformants seemed to have the similar plant height, size and shape of leaves, and inflorescence development as wild-type plants (Figs. 1 A, B). However, the corolla was significantly smaller (Figs. 1 C, D) in 10 of the sense-*WAG* transformants (Table 1). One transformant with the antisense *WAG* (*WAG*-antisense 11) had a larger corolla than that of the wild type (Table 1). The vertical and horizontal diameters of the lip part of the corolla, as well as the length of the corolla (including the tube part), were all reduced on most of the smaller flowers (Table 1), suggesting that the corollas were reduced equally in all dimensions.

With the exception of corolla size, the formation of the floral organs seemed normal. Seeds were obtained from the plants with small corollas after self-fertilization, but the seeds set at a lower rate than did those of wild-type plants (data not shown). Introduction of the sense-*WAG* seemed to disturb some function of the stamen and/or pistil.

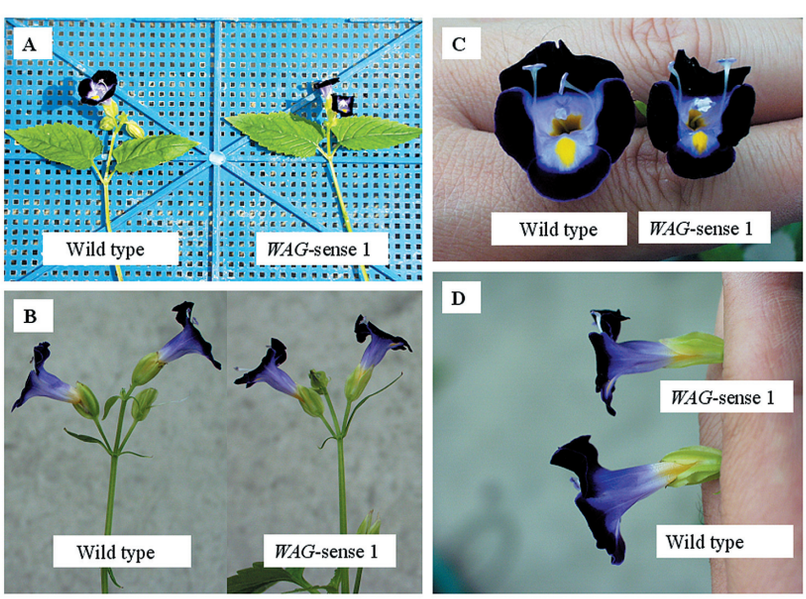

Figure 1.
Phenotypic changes in the torenia transformants with the sense *WAG* gene (*WAG*-sense 1).
All the transformants had the similar plant height, size and shape of leaves (A), and inflorescence development (B) as wild-type plants. However, the corollas of some transformants with the sense *WAG* were markedly smaller than those of the wild type (C, D).

Table 1. Size of corollas in transformants (average±S.D.)[z)]

Line	Vertical diameter of lip (mm)		Horizontal diameter of lip (mm)		Length of the corolla (mm)		Ploidy level
Wild type	24.0 ± 1.4		22.2 ± 0.8		22.8 ± 1.3		2x
WAG-sense 1	18.8 ± 2.9	*y) S[x)]	18.8 ± 1.8	*y) S[x)]	20.0 ± 1.2	*y) S[x)]	2x
2	25.0 ± 1.6		23.0 ± 0.7		23.2 ± 0.4		2x
3	24.6 ± 1.5		23.0 ± 1.2		23.4 ± 0.5		2x
4	21.2 ± 1.3	* S	19.8 ± 1.6		21.6 ± 2.2		2x
5	20.2 ± 1.3	** S	19.4 ± 1.5	* S	21.0 ± 1.7		2x
6	20.0 ± 1.4	** S	19.0 ± 0.7	** S	20.2 ± 1.3	* S	2x
7	21.8 ± 1.6		20.6 ± 1.5		21.0 ± 1.2		2x
8	22.2 ± 2.2		21.6 ± 2.3		21.6 ± 1.5		2x
9	20.0 ± 1.0	** S	19.8 ± 1.3	* S	20.0 ± 1.4	* S	2x
10	19.0 ± 1.7	** S	17.2 ± 1.5	** S	18.8 ± 1.5	** S	2x
11	18.6 ± 1.7	** S	18.8 ± 1.5	** S	18.8 ± 2.8		2x
12	21.2 ± 1.9		19.0 ± 2.6		20.2 ± 1.3		2x
13	19.6 ± 2.1	** S	18.4 ± 1.3	** S	19.6 ± 1.5	* S	2x
14	24.4 ± 1.5		24.2 ± 1.5		24.0 ± 1.2		2x
15	18.6 ± 1.1	** S	18.0 ± 1.2	** S	16.4 ± 3.3	* S	2x
16	22.8 ± 0.8		21.0 ± 1.0		22.0 ± 1.2		2x
17	23.2 ± 0.8		21.4 ± 1.5		22.0 ± 1.2		2x
18	20.6 ± 0.5	** S	19.6 ± 0.5	** S	20.0 ± 0.7	** S	2x
19	22.8 ± 1.3		20.8 ± 1.3		21.6 ± 2.1		2x
20	23.0 ± 1.2		21.6 ± 1.1		23.0 ± 1.0		2x
21	23.0 ± 0.7		21.4 ± 0.5		22.4 ± 0.5		2x
WAG-antisense 1	25.4 ± 1.5		23.0 ± 1.9		23.2 ± 0.8		2x
2	24.0 ± 1.2		21.6 ± 0.5		22.0 ± 0.7		2x
3	23.4 ± 1.1		21.4 ± 1.1		22.8 ± 1.9		2x
4	21.4 ± 1.7		20.6 ± 1.3		21.6 ± 0.9		2x
5	25.8 ± 1.6		24.2 ± 1.3		23.8 ± 0.8		2x
6	24.0 ± 1.0		23.0 ± 0.7		22.6 ± 0.5		2x
7	23.6 ± 1.1		21.8 ± 0.8		21.2 ± 1.1		2x
8	23.5 ± 1.3		21.8 ± 1.3		21.8 ± 0.8		2x
9	23.2 ± 1.5		22.0 ± 1.6		22.0 ± 0.7		2x
10	25.2 ± 1.3		23.6 ± 1.3		22.4 ± 1.3		2x
11	33.0 ± 3.0	** L	30.6 ± 2.5	** L	24.0 ± 1.6	** L	4x
12	23.8 ± 1.3		22.0 ± 1.2		21.8 ± 1.1		2x

[z)] Vertical and horizontal diameters of the lip part of the corolla and length of the corolla. Five corollas were examined in each line.
[y)] Significantly different from wild type (** = 1% probability, * = 5% probability).
[x)] S: shorter than wild type, L: longer than wild type.

Southern blot analysis

Southern blot analysis showed that, of all the sense-*WAG*-introduced transformants examined, four plants with reduced corolla size (*WAG*-sense 1, 5, 11 and 18) and four plants with normal corollas (*WAG*-sense 7, 16, 19 and 20) possessed the *WAG* gene in their genomes (Fig. 2). We hypothesized that the level of expression of the introduced *WAG* gene would be different in each transformant, causing of the variation in corolla size.

There was a faint band on all the transformants and wild-type plants, demonstrating that torenia has no endogenous DNA sequences with high homology to the *WAG* gene but a DNA sequences with low homology. Because there is no endogenous gene that is highly *WAG*-homologous in torenia, introduction of the antisense-*WAG* would not affect any expression of

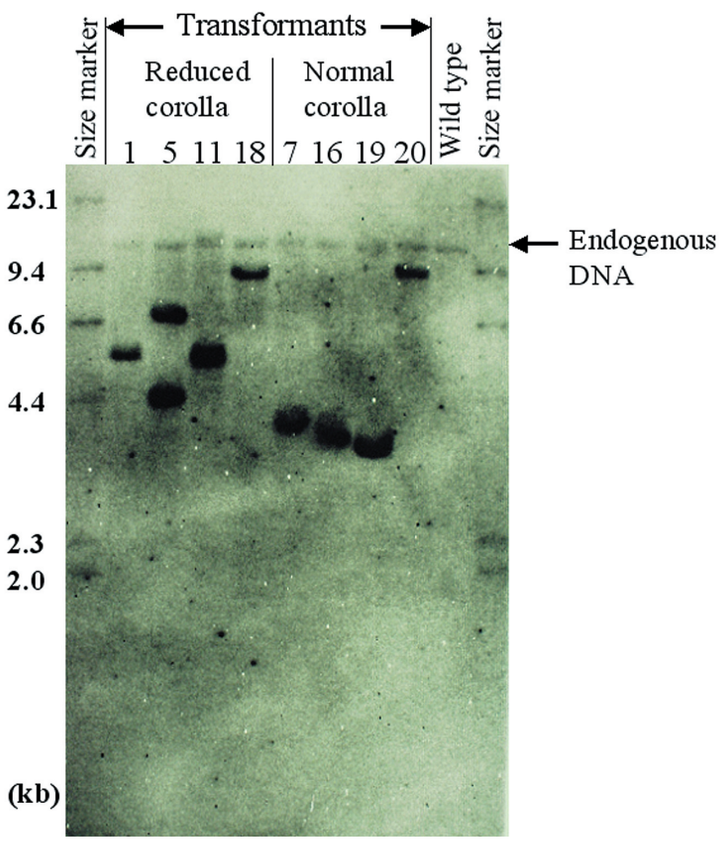

Figure 2.
Southern blot analysis of torenia transformants with the sense *WAG* gene.
All the transformants examined – four with small corollas (*WAG*-sense 1, 5, 11 and 18) and four with normal-sized ones (*WAG*-sense 7, 16, 19 and 20)– had extra band(s) compared with wild type plants. There was a faint band on all the samples, suggesting that torenia has, at least, an endogenous DNA sequences with low homology to *WAG*.

endogenous genes. It is reasonable that no characteristic change was observed in the antisense-*WAG* transformants.

Ploidy level of transformants

Chromosomal changes have been observed in many species of plants regenerated through tissue culture. The most common chromosomal change is increase in ploidy level, by chromosomal doubling (Skirvin 1978). Straub (1939) reported that torenia plants with a 4x ploidy level had larger corollas than did normal 2x plants. We examined ploidy levels among the transformants. One transformant with larger corolla (*WAG*-antisense 11) showed a ploidy level of 4x and all of the

other transformants had a ploidy level of 2x (Table 1). This result suggested that the reduction in corolla size was linked to introduction of the sense-*WAG* and the increase in corolla size was caused by polyploidization of the transformants.

WAG

Function of in torenia

Introduction of the *WAG* gene for the 1.1-kb transcript with sense orientation reduced the corolla size in torenia, whereas the structure of the floral organs was not modified. Similar alterations have been previously reported in transgenic petunia. Ectopic expression of *FBP6* (*Floral Binding Protein gene 6*, a petunia *AGAMOUS* homolog) results in reduced petal size with normal floral structure in petunia (Kater et al. 1998). In transgenic *Arabidopsis*, ectopic expression of the *Lily MADS box gene 1*, an *AP3* family gene, produced flowers with short petals and stamens, but no floral organ conversion was observed (Tzeng and Yang 2001). Ectopic expression of the *Arabidopsis NAP* (*NAP-LIKE, ACTIVATED BY AP3/PI*) gene also produced *Arabidopsi*s flowers with short petals and stamens, by inhibiting cell expansion specifically in the flowers (Sablowski and Meyerowitz 1998). These reports suggest that some genes related to floral homeotic genes might be involved in increasing the size of floral organs, mainly the petals. The *WAG* gene for the 1.1-kb transcript reduced the size of the corolla in torenia, but the mechanism of formation of this smaller corolla is not yet clear. Further investigations would clarify the kind of interaction that occurs between the 1.1-kb *WAG* transcript and other genes, and would help us determine how the size of the torenia corolla is reduced.

The 1.1-kb *WAG* transcript has been observed in vegetative tissues as well as in floral organs in wheat (Meguro et al. 2003). In torenia, the *WAG* sense transgene with the 35S promoter should be expressed in the vegetative tissues as well as in the floral organs, but no characteristic change was observed in the vegetative tissues (leaves and stems) of the transgenic torenia plants. We were unable to shed light on the function of the *WAG* gene for the 1.1-kb transcript in the vegetative tissues, but this new knowledge of the function of the *WAG* gene in reducing corolla size in torenia might give us clues as to its proper function in wheat.

Acknowledgment

We thank Kumiko Bessho and Setsuko Hosoda for the transformation of torenia.

Literature Cited

Aida, R. and M. Shibata. 2001. Transgenic *Torenia fournieri* Lind. (torenia). *In* "Biotechnology in Agriculture and Forestry, Vol. 48, Transgenic Crops Ⅲ " Bajaj, Y.P.S. (ed.), Springer-Verlag, Berlin, Heidelberg. p. 294–305.

Aida, R., S. Kishimoto, Y. Tanaka and M. Shibata. 2000. Modification of flower color in torenia (*Torenia fournieri* Lind.) by genetic transformation. Plant Sci. 153: 33–42.

Coen, E.S. and E.M. Meyerowitz. 1991. The war of the whorls: genetic interactions controlling flower development. Nature 353: 31–37.

Kater, M.M., L. Colombo, J. Franken, M. Busscher, S. Masiero, M.M. van Lookeren Campagne and G.C. Angenent. 1998. Multiple *AGAMOUS* homolog from cucumber and petunia differ in their ability to induce reproductive organ fate. Plant Cell 10: 171–182.

Meguro, A., S. Takumi, Y. Ogihara, K. Murai. 2003. *WAG*, a wheat *AGAMOUS* homolog, is associated with development of pistil-like stamens in alloplasmic wheats. Sex. Plant Reprod. 15: 221–230.

Murai, K., S. Takumi, H. Koga and Y. Ogihara. 2002. Pistillody, homeotic transformation of stamens into pistil-like

structures, caused by nuclear-cytoplasm interaction in wheat. Plant J. 29: 169–181.

Sablowski, R.W.M and E.M. Meyerowitz. 1998. A homolog of *NO APICAL MERISTEM* is an immediate target of the floral homeotic genes *APETALA3*/*PISTILLATA*. Cell 92: 93–103.

Skirvin, R. M. 1978. Natural and induced variation in tissue culture. Euphytica 27: 241–266

Straub, J. 1939. Chromosomenuntersuchungen an polyploiden Blütenpflanzen. I. Die Chromatinmasse bei künstlich ausgelösten Autopolyploiden. Ber. Deutsch. Bot. Ges. 57: 531–544

Theissen, G., A. Becker, A. Rosa, A. di. Kanno, J.T. Kim, T. Munster, K.U. Winter and H. Saedler. 2000. A short history of MADS-box genes in plants. Plant Mol. Biol. 42: 115–149.

Tsunewaki, K. 1989. Plasmon diversity in *Triticum* and *Aegilops* and its implication in wheat evolution. Genome 31: 143–154.

Tzeng, T.-Y. and C.-H. Yang. 2001. A MADS box gene from lily (*Lilium longiflorum*) is sufficient to generate dominant negative mutation by interacting with *PISTILLATA* (*PI*) in *Arabidopsis thaliana*. Plant Cell Physiol. 42: 1156–1168.

コムギの *AGAMOUS* 相同遺伝子 *WAG* を導入したトレニアの花冠は小さくなる

間竜太郎・村井耕二・岸本早苗・大宮あけみ

和文摘要

コムギの *WAG*（シロイヌナズナ *AGAMOUS* の相同遺伝子）の 1.1kb 転写物の機能は明らかでない．そこで，1.1kb 転写物をコードする *WAG* をトレニア（*Torenia fournieri*）に導入し，形態の変化を調査することで機能の推定を試みた．CaMV35S プロモーターに *WAG*(1.1kb) をセンスあるいはアンチセンス方向に連結し，アグロバクテリウム法によりトレニアに導入した．形質転換体（センス；21 個体，アンチセンス；12 個体）は隔離温室で栽培し，花器官等の形態の変化を観察した．形質転換体の開花に至るまでの生育は野生型植物と同様であり，節間長や葉の形態・大きさ，花序，開花時期についても両者の間に差は認められなかった．ところが，開花した花を観察したところ，センス遺伝子を導入した 21 個体の内 10 個体において，有意に花冠が小さくなっていた．これらの植物においては，唇形部の縦方向，横方向及び筒状部を含む奥行きのいずれの長さも短くなっており，花冠全体が小型化していた．なお，がく，雄ずい，雌ずいの形態については差は認められなかった．なお，サザンブロット分析によりトレニア形質転換体における導入 *WAG* 遺伝子の存在を確認した．これらの結果から，コムギの *AGAMOUS* 相同遺伝子 *WAG*(1.1kb) はトレニアの花冠を小さくする機能を有することが明らかとなった．